

Appl. No. : 09/979,545
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AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 5, line 1 as follows:

Also, a kit for identifying DNA mutation comprises a microwell whose inside has amine group, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) solution and 1-methylimidazole solution, pH 7.0 for affixing a probe, 0.4M NaOH/0.25% ~~tween-20~~ TWEEN-20TM solution for removing unaffixed probe, a solution containing dH₂O, 20xSSPE/0.0167% ~~triton X-100~~ TRITON X-100TM and salmon sperm DNA (10mg/ml) for blocking microwell surface, a solution of 0.5xSSC/0.1% % ~~tween-20~~ TWEEN-20TM for removing unhybridized biotinylated sample DNA fragments, a streptavidin-linked degradation enzyme to be bound to biotin, 100mM Tris-HCl (pH 7.5) solution containing 150mM NaCl for binding of streptavidin to biotin, 100mM Tris-HCl (pH 7.5) solution containing 150mM NaCl/0.1% % ~~tween-20~~ TWEEN-20TM for removing unbound streptavidin, and a substrate for the streptavidin-linked degradation enzyme.

Please amend the paragraph beginning at page 6, line 26 as follows:

The probes thus prepared are affixed to amine group on the inner surface of microwell: Since nucleotide sequences of synthesized probe may be reannealed spontaneously, the single stranded probes can be maintained by heating the probes for 5 to 15 minutes, most preferably for 10 minutes, at 90 to 100°C, most preferably at 94°C, followed by immediate cooling in ice water. Ice cold solutions of 10mM 1-methylimidazole, pH 7.0 and 10mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), pH 7.0 which catalyze the covalent-binding reaction of phosphate moiety of oligonucleotide and amine group on microwell are added to the single-stranded DNA probe. The probe-containing mixture was added to the amine-derivatized microwell, and the microwells are sealed with tape to prevent evaporation of reaction mixture, followed by incubation for 5 to 9 hours, most preferably for 7 hours, at 40 to 60°C, most preferably at 50°C. In this way, the probes are covalently affixed on the inner surface of microwell. Probe-affixed microwells are washed with 0.4M NaOH/0.25% ~~tween-20~~ TWEEN-20TM, and then with distilled water at room temperature.

Please amend the paragraph beginning at page 7, line 13 as follows:

Biotinylated PCR fragments obtained in the previous step are added to the probe-affixed microwells: First, a solution containing dH₂O, 20xSSPE/0.0167% ~~triton X-100~~ TRITON X-100TM and salmon sperm DNA (10mg/ml) is added to microwells, and incubated for 10 to 20 minutes, most preferably for 15 minutes, at 40 to 60°C, most preferably at 50°C, to prevent biotinylated sample from binding to free amine group on the microwell surface. In order to denature biotinylated double-stranded DNA fragments obtained in Step 1 to allow hybridization with the probe, they are heated for 5 to 15 minutes, most preferably for 10 minutes, at 90 to 98°C, most preferably at 94°C, and chilled immediately in ice water. And then, a solution containing dH₂O,

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20XSSPE/0.0167% ~~triton X-100~~ TRITON X-100™ and salmon sperm DNA (10mg/ml) is added to the single-stranded DNA sample. The mixture is introduced into the microwells, incubated for 5 to 15 hours, most preferably for 10 hours, at 50 to 70°C, most preferably at 60°C. After incubation, the residual mixture is removed, and microwells are washed with 0.5xSSC, 0.1% ~~tween-20~~ TWEEN-20™. The sample with changed nucleotide sequence(s) would show low affinity for the probe affixed to microwell while the sample with normal sequence would show high affinity. Accordingly, biotin of the sample DNA fragments can be captured to the microwell, and the affinity difference between mutated and non-mutated sequences for the probe can be measured in terms of the difference in density of microwell-bound biotin.

Please amend the paragraph beginning at page 8, line 6 as follows:

Streptavidin-linked degradation enzyme is added to the microwell in order to bind the degradation enzyme to biotin moiety of the probe-captured sample DNA fragment: To detect the difference in biotin density bound to the microwell of mutated and non-mutated DNA samples, degradation enzyme is bound to biotin. The degradation enzyme hydrolyzes a chromogenic substrate, and resulting changes in color intensity or absorbance can be measured. For example, streptavidin-alkaline phosphatase can be used; microwells are washed with a buffer solution of 100mM Tris-HCl (pH 7.5) containing 150mM NaCl/0.1% ~~tween-20~~ TWEEN-20™, and streptavidin-alkaline phosphatase diluted with a buffer solution of 100mM Tris-HCl (pH 7.5) containing 150mM NaCl, is added to the microwell, and incubated for 30 to 90 minutes, most preferably for 60 minutes, at 35 to 45°C, most preferably at 40°C. After removal of the residual reaction mixture, a buffer solution of 100mM Tris-HCl (pH 7.5) containing 150mM NaCl/0.1% ~~tween-20~~ TWEEN-20™ is added, incubated for 5 to 15 minutes, most preferably for 10 minutes, at 50 to 70°C, most preferably at 60°C, and then washed.

Please amend the paragraph beginning at page 9, line 8 as follows:

Also, the present invention provides a kit comprising the following components to implement the method described above: microwell whose inside has amine group, EDC solution and 1-methylimidazole solution, pH 7.0 for affixing a probe, 0.4M NaOH/0.25% ~~tween-20~~ TWEEN-20™ solution for removing unaffixed probe, a solution containing dH₂O, 20x SSPE/0.0167% TRITON X-100™ and salmon sperm DNA (10mg/ml) for blocking free amine groups on microwell surface, a solution of 0.5xSSC/0.1% ~~tween-20~~ TWEEN-20™ for removing unhybridized biotinylated sample DNA fragments, a streptavidin-linked degradation enzyme to be bound to biotin, 100mM Tris-HCl (pH 7.5) solution containing 150mM NaCl for binding of streptavidin to biotin, 100mM Tris-HCl (pH 7.5) solution containing 150mM NaCl/0.1% ~~tween-20~~ TWEEN-20™ for removing unbound streptavidin, and a substrate for the streptavidin-linked degradation enzyme.

Please amend the paragraph beginning at page 12, line 15 as follows:

Subsequently, probe #1 comprising a normal nucleotide sequence of 5'-

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TTGACCTCGCCAGGAGAGAAGATCA-3' (SEQ ID: 3) and probe #2 comprising a normal nucleotide sequence of 5'-TCCGTACGCTCGAAACGCTTCCAAC-3') was synthesized. 10pmol aliquots of each probe were heated for 10 minutes at 94°C, cooled down for 10 minutes in ice water, condensed by centrifugation. And then, a solution of EDC and 1-methylimidazole pH 7.0 was added to each aliquot of the probe to a final concentration of 10mM. 100µl of each probe mixture thus prepared was introduced into microwells (Nunc, Denmark) on ice, incubated for 7 hours at 50°C. After removing the residual probe mixture, a solution containing 138µl of dH₂O, 20xSSPE/0.0167% ~~triton X-100~~ TRITON X-100™, and 2µl of salmon sperm DNA (10mg/ml) was added to the microwells, and incubated for 20 minutes at 50°C for blocking free amine group on the microwell surface.

Please amend the paragraph beginning at page 12, line 31 as follows:

After removing the blocking solution, a sample mixture containing 68µl of dH₂O, 30µl of 20xSSPE/0.0167% ~~triton X-100~~ TRITON X-100™, 1µl of salmon sperm DNA (10mg/ml) and 1µl of PCR amplified sample DNA prepared above was added to each microwell, and incubated for about 10 hours at 60°C. After removing the mixture, each microwell was washed 3 times with 200µl of 0.5xSSC, 0.1% ~~tween-20~~ TWEEN-20™, and the same solution was added to incubate for 15 minutes at 60°C.

Please amend the paragraph beginning at page 13, line 7 as follows:

Subsequently, the microwells were washed 3 times with a buffer solution of 100mM Tris-HCl (pH 7.5) containing 150mM NaCl/0.1% ~~tween-20~~ TWEEN-20™, and subjected to 3 times of incubation for 5 minutes at 60°C and washing with the same solution. 100µl of pNPP (N7653, SIGMA, U.S.A.) was added to each well, incubated for 90 minutes, and then the absorbance was measured at 405nm (see: Figure 1).